

Gender and Risk of Autoimmune Diseases: Possible Role of Estrogenic Compounds

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A striking common feature of many autoimmune diseases in humans and experimental animals, despite differences in pathology, is that females are highly susceptible to autoimmune conditions compared to males. In several animal models, estrogens promote, whereas androgens abrogate, B-cell-mediated autoimmune diseases. To understand mechanisms by which estrogens regulate autoimmunity, it is first necessary to decipher estrogen effects on the normal immune system. Estrogen treatment of nonautoimmune mice diminished lymphocyte numbers in both developmental and mature lymphoid organs. Estrogen dysregulated T- and B-cell balance by inducing selective T-cell hypoactivity and B-cell hyperactivity. Even though estrogen did not alter the relative percentages of splenic T-cell subsets, splenic lymphocytes had a reduced proliferative response to T-cell stimulants and were refractory to rescue from activation-induced apoptosis compared to cells from placebo-treated mice. In contrast, estrogen induced B-cell hyperactivity (promoted autoantibodies to double-stranded DNA and phospholipids, increased numbers of plasma cells, and increased autoantibody yield per B cell). Note that treatment of normal mice with estrogen can alter T- and B-cell regulation and overcome B-cell tolerance to result in autoimmunity in normal individuals. Could environmental estrogens promote some human autoimmune disorders? Is there a link between environmental estrogens and autoimmune disorders, especially since these disorders are reported possibly more frequently? These provocative questions warrant investigation. Our findings on immunomodulatory effects may serve as a benchmark to examine whether endocrine-disrupting chemicals will have similar immunologic effects. **Key words:** autoantibodies, autoimmune diseases, autoreactive cells, B cells, diethylstilbestrol, endocrine-disrupting chemicals, estrogens, T cells. — *Environ Health Perspect* 107(suppl 5):681–686 (1999). <http://ehpnet1.niehs.nih.gov/docs/1999/suppl-5/681-686ahmed/abstract.html>

It is evident that there are marked or subtle gender differences in the functioning of several nonreproductive tissues. There are thousands of research manuscripts that have pointed out gender differences in various tissues and systems, including the immune system. The immune system serves as an archetypal example of gender differences in nonreproductive sites (see “Gender Differences in Immune Responses”). Given the marked impact of gender on tissue physiology, it is imperative to include gender as a variable in clinical and experimental studies. Nevertheless, many research protocols do not include study subjects of both genders nor do they strictly control for gender (unlike importance given to age or strain), an aspect that warrants attention.

Females as a gender group have heightened immune responses not only to foreign antigens but also to self-antigens [reviewed in (1–6)]. Thus, there is a greater preponderance of autoimmune disorders in women than in men [see “Gender Differences in Immune Responses”; also reviewed in (1–5,7)]. It is important to recognize that autoimmune diseases can afflict almost any tissue, akin to cancer. Conceivably, the initiating and pathogenic mechanisms are most likely to be different in various types of

autoimmune diseases. Yet, in general, women are more susceptible than men to autoimmune diseases. Therefore, gender is a common thread that stitches together the disparate autoimmune conditions.

Gender Differences in Immune Responses

In general, females as a gender group have better B-cell-mediated immunity (often, perhaps incorrectly, referred to as humoral immunity) than age-matched male counterparts [reviewed in (1–6)]. They have higher immunoglobulin levels, stronger antibody responses to various foreign antigens, and increased resistance to certain infections [reviewed in (1–6)]. Gender differences in T-cell-mediated immunity also exist, although the gender influences appear to be complex [reviewed in (1–6,8)]. Females have greater resistance to induced tolerance, increased ability to reject grafts, and increased CD4 to CD8 ratios. Also, females tend to secrete higher levels of interleukin (IL)-4, interferon- γ (INF- γ), and IL-1. Sex hormonal action on the immune system is thought to account for gender differences in immune capability, dispelling the notion that sex steroid hormones exclusively affect sex-related endocrinologic functions.

Sources of Exposure to Estrogens: Natural, Synthetic, and Environmental Estrogens

In addition to natural estrogens, human and animals are exposed to estrogens in different forms. The exposure to synthetic and environmental estrogens is believed to have increased over the years. Diethylstilbestrol (DES), a potent synthetic estrogen, has been used as a therapeutic agent in a variety of clinical situations including threatened abortions, preeclampsia, prior premature labor, prostatic and breast cancer, pregnancy complications in diabetic women, and whenever estrogen-replacement therapy was indicated (9–11). Although DES is no longer used during pregnancy, it is estimated that 2–4.8 million human offspring were exposed to DES from the 1940s through 1971 (12). Human exposure to DES is suspected to have also occurred through consumption of meat and milk products of livestock that were fed DES as a food additive. Moreover, there was an occupational exposure to farmers handling DES (13). Estrogens are now increasingly prescribed for postmenopausal women for prevention of bone loss and cardiac myopathies. Further, a greater number of women worldwide are on prolonged estrogen-based oral contraceptives. Finally, an increasing number of people are unintentionally exposed to a wide range of synthetic chemicals such as pesticides, industrial byproducts, insecticides, fungicides, and herbicides that have estrogenic or antiestrogenic effects (13–15). Food animals are likely to have been exposed to estrogenic zearalanol and/or zearalenone, an estrogenic *Fusarium*-derived mycotoxin in contaminated diet. It is likely that a subset of

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the population that may be exquisitely sensitive to estrogenic compounds may be at risk for the development of autoimmune diseases.

Gender Difference in Autoimmune Diseases: The Role of Estrogenic Compounds
Human Studies

Genetic, viral, stress, and sex hormonal factors all play a complex role in the etiology of autoimmune diseases. For the first three of these four factors, despite intense research activity and exciting new data, their precise contribution to autoimmune disease is not yet clear. For sex hormones, however, the clinical observations that autoimmune diseases occur more frequently in females compared to males offers exciting new avenues of research to better understand the promotion, progression, and possible treatment of autoimmune diseases. We and others have extensively documented gender differences in the expression of autoimmune diseases in previous reviews (1–5,7). A partial list of female predominance of autoimmune diseases is shown in Table 1. [See Ansar Ahmed et al. (1) for a more detailed list of gender differences.] A recent epidemiologic study on the prevalence of 24 autoimmune diseases estimated that 1 in 31 Americans (almost 9 million), mostly women, are afflicted with some type of autoimmune disease (16). Of the autoimmune diseases studied, thyroid autoimmune disorders, rheumatoid arthritis, insulin-dependent diabetes, and pernicious anemia were common autoimmune disorders. Most of these disorders predominantly afflict women. Although individual autoimmune disease may be relatively uncommon, collectively they constitute a significant concern to internal

medicine. Autoimmune diseases are believed to be more frequently reported, which may be due to better recognition of these diseases by physicians and patients, coupled with more sensitive diagnostic procedures. Nevertheless, the possibility that there is a true increase in autoimmune disease cannot be completely discounted. Could the possible increase in the incidence of autoimmune diseases be due to exposure to environmental estrogens? This provocative question needs to be answered in future studies, especially with regard to the impact of endocrine-disrupting chemicals on disease initiation or progression.

The influence of sex hormones on human autoimmune diseases is evidenced by the observation that the course of many of these disorders is modulated during the periods of sex hormonal alterations (e.g., pregnancy, administration of oral contraceptives, or menopause) [reviewed in (1–5,7)]. Limited data in humans have shown that dehydroepiandrosterone therapy in systemic lupus erythematosus (SLE) patients (17) or androgen replacement therapy in a Klinefelter syndrome patient with SLE (18) had apparent beneficial effects.

Women prenatally exposed to DES are susceptible to the development of adenocarcinomas of the cervix and vagina (9–12,19) and reproductive abnormalities [vaginal adenosis and infertility (9,12)]. Further, a link between prenatal DES exposure and autoimmune disease has been suggested (20,21). Women prenatally exposed to DES appear to have a higher incidence of autoimmune diseases but only when various autoimmune disorders are grouped (20,21). Women with vaginal epithelial changes had nearly 50% more autoimmune diseases than DES-exposed women lacking these

pathologic changes (20). The DES–adenosis report (20) stated

The information presented in this preliminary communication also supports the concept that human exposure before birth to DES may subsequently affect the adult immune system. Additional studies should be undertaken to thoroughly investigate the function of the immune system in DES-exposed women by means of appropriate serologic testing.

An epidemiologic questionnaire-based study involving 1,700 respondents (including mothers, daughters, sons) revealed that respiratory infections (flu, colds), asthma, arthritis, and lupus were reported more frequently (21). This suggests that prenatal DES exposure contributed to immune impairment. Another recent but smaller preliminary study involving self-reported cases of allergy, infections, and autoimmune diseases did not find an association of these disorders with prenatal DES exposure (22). Nevertheless, these authors felt further studies are needed to conclusively establish the link between prenatal DES exposure and autoimmune diseases. Large double-blind human studies are needed to address this aspect.

Animal Studies

Akin to the human situation, gender differences in autoimmune diseases and/or sex hormonal influences on the expression of these disorders are evident in many animal models of autoimmune diseases [Table 1; (1–5)]. Space restrictions do not permit comprehensive discussion of literature and therefore only selected recent information is included here. A relatively new murine model for SLE has been described in female (SWR × SJL)F₁ mice. These mice have increased hypergammaglobulinemia, a 2-fold increase in spleen cell numbers, and make anti-Sm/U1snRNP antibodies (which react with autogenic peptides), compared to males. IgG antibodies react with PPPGMRPP, an octapeptide thought to be a strong immunoreactive linear epitope in SLE patients with anti-Sm/U1snRNP antibodies (23). The increased susceptibility of female (SWR × SJL)F₁ mice to lupus resembles the differential sex susceptibility in (NZB × NZW)F₁, or B/W, and (NZB × SWR)F₁, or SNF₁, mice. In B/W mice, depletion of male hormones (by administration of estrogens or antiandrogens to orchietomized males) accelerates the manifestation of the disease (1–5, 24,25). Conversely, female B/W mice can be made resistant to the disease by administration of androgens or antiestrogens (1–5,24). In SNF₁ mice, resistant males were rendered susceptible by estradiol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) treatment (26). Estrogen-induced exacerbation of

Table 1. A partial list of gender differences in human and experimental autoimmune disease.

Human		Animal models	
Autoimmune disease	Female to male ratio	Autoimmune disease	Female to male ratio
Autoimmune thyroiditis		Autoimmune thyroiditis	
Hashimoto thyroiditis	25:1	Spontaneous (BUF rats)	3:1
Graves-Basedow disease	4 to 8:1	Induced	
		Thymectomy-irradiation	4 to 6:1
		Neonatal thymectomy	4:1
		Chemical and autoantigen	2:1
SLE	9 to 13:1	SLE	Earlier expression of
Sjögren syndrome	9:1	Rheumatoid arthritis and/or	autoimmune disease
		Sjögren syndrome	or autoantibodies in
			females, e.g.,
			(NZB × NZW)F ₁ , SNF ₁ ,
			(NZB × DBA/2)F ₁ ,
			(NZB × DBA)F ₁ ,
			MRL/lpr/lpr
Rheumatoid arthritis	2:1		
Juvenile onset of myasthenia gravis			
(White patients)	2 to 14:1		
(Black patients)	1:1 (approx)		
Scleroderma	3 to 4:1	Polyarthritis (LEW rats)	6:1

SLE, systemic lupus erythematosus. Modified from Ansar Ahmed et al. (1).

autoimmune disease is thought to be mediated through alterations in T cells (increased CD4⁺ cells in the thymus and CD4⁺ memory T cells in the spleen and kidneys) (26). Estrogen promotion of autoimmune disease has also been shown in MRL/lpr/lpr mice, (which develop an aggressive disease with lymphadenopathy) (27), and in an immunized model for SLE (16/6 idiotype bearing anti-DNA antibodies) (28). In the nonobese diabetic strain of mouse, the incidence of spontaneous diabetes is increased in females compared to males. The susceptibility of males is augmented by orchiectomy (29). Orchiectomy combined with thymectomy considerably enhances the incidence of the disease, suggesting that male hormones may act via the gonadal-thymic axis. In a mouse model of arthritis, female mice have a greater ability to degrade implanted cartilage than males (30). Granulomata from female mice had higher levels of IL-1, implying that sex hormone modulation of cartilage destruction may be mediated by cytokines. In experimental autoimmune encephalomyelitis in SJL mice injected with myelin basic protein-specific T cells, dihydrotestosterone was effective in diminishing the severity of the disease (31). The protective effects apparently are related to enhanced production of IL-10 by antigen-specific T lymphocytes.

Mice given DES during the perinatal (neonatal) period have an impaired immune system (particularly T cells), including atrophy of the thymus, reduced proliferative response to T-cell mitogens, diminished antigen-specific delayed-type hypersensitivity response and graft versus host reaction, decreased cytotoxic response to mammary tumor virus, and decreased natural killer cell function (32–36). Therefore, concerns have been raised that the exposure of fetuses to estrogenic compounds during the highly critical stage of immune development may alter the immune system. We are currently investigating the immune consequences of prenatal exposure to DES in a murine model. These studies may also be relevant to other endocrine-disrupting chemicals (e.g., octylphenol or genestein).

The following section describes studies from our laboratory as well as others on the immune effects of estrogen in normal animals. Extensive studies in our laboratory have identified immune cells that are modulated by estrogen.

Immune Biomarkers for Estrogenic Compounds

Effects on Thymus and T Cells

To better understand how sex hormones regulate autoimmune responses and to identify the target organs and cells, it is essential to first decipher their effects on normal

immune systems, an aspect thus far not well understood. Toward this aim, we gave estrogen (17 β -estradiol in silastic capsules) to prepubertal orchiectomized normal C57BL/6 mice and examined their splenic and thymic tissues after 3–6 months (37–40). Extensive studies from our laboratory as well as others have established immune biomarkers for estrogen [Table 2; (37–41)]. These parameters may be useful in ascertaining the immunologic consequences of exposure to endocrine disrupters. Chronic estrogen treatment diminished lymphocyte numbers in both developmental (thymus, bone marrow) and mature (spleen, lymph nodes) lymphoid organs. Estrogens (presumably including DES) induced atrophy of the thymus. Estrogen also altered intrathymic T-cell subsets (e.g., loss of CD4⁺8⁺ thymocytes) (Figure 1). To address whether estrogenic compound-induced atrophy of the thymus is due to direct induction of apoptosis of thymocytes, normal thymocytes were cultured with various doses of DES and the kinetics of apoptosis was determined. Parallel cultures of thymocytes were exposed to a known thymic atrophy- and thymocyte apoptosis-inducing hormone, dexamethasone (DEX). Apoptosis was evaluated by flow cytometric examination of cells stained with propidium iodide (PI), 7-aminoactinomycin D (7-AAD), or fluorescein isothiocyanate-annexin V; by forward/side scatter analysis, cell-size analyzer, and cytopathologic examination. In our extensive studies, apoptosis could only be detected in thymocyte cultures exposed to DEX but not in those exposed to DES (41). These studies imply that these two synthetic hormones induce atrophy of the thymus by dissimilar mechanisms *in vivo*. A recent study using transgenic mice overexpressing antiapoptotic oncogene *bcl-2* has shown that administration of 17 β -estradiol, but not DEX, can induce thymic atrophy, thereby suggesting that these two hormones have different modes of action (42). Alternatively, thymic injury mediated by

estrogen or DES may be due to diminished immigration of prothymocytes from the bone marrow or hormonal effects on thymic stromal cells (possibly by altering their secretion of crucial cytokines such as IL-1, IL-6, or IL-7, or by inducing apoptosis of these cells). Sex steroid receptors have been demonstrated on thymic epithelial/stromal cells, possibly on thymocytes, and on peripheral T-cell subsets in a number of species [reviewed in (1–6)]. A putative model suggests that sex steroid hormones bind to intracellular receptors (nuclear or possibly in the cytosol) in lymphocytes; this complex binds to sex steroid hormone-responsive elements in the DNA to modify cellular activity. Sex hormones induce the thymus to release immunoregulatory thymic factors that can also act on the hypothalamus–pituitary axis to release other immunoregulatory peptides [reviewed in (1–6)]. Studies also suggest that developing thymus and T cells are direct or indirect targets for estrogenic compounds. Induction of a hyperestrogenic state induced by estrogen treatment of pregnant guinea pigs or DES injections of pregnant mice resulted in reduction in the number of large cortical cells of the fetal guinea pig thymus (43) and lowered response to T-cell mitogens (27). Our ongoing studies show that nonautoimmune mice prenatally exposed to DES have altered thymocyte numbers or functions (44). Thus, it is conceivable that exposure to estrogenic compounds during fetal life could be a potential immunologic

Table 2. Immune biomarkers for estrogen.

Hyperactivity of B cells
↑ Immunoglobulins and autoantibodies
↑ Number of plasma cells
↑ Output of autoantibodies per B cell
↑ Number of cells in the S phase of cell cycle
↑ Survival of activation-induced apoptosis
Impairment of T-cell function
↓ Proliferative response to T-cell stimulants
Unresponsive to activation signals for rescue from apoptosis
↓ CD69 expression after T-cell stimulation
↑ IFN- γ at protein and mRNA levels
Dampened NK cell activity
Increased granulocytes
↑ Ly6G ⁺ (Gr-1)

Abbreviations: IFN- γ , interferon- γ ; NK, natural killer.

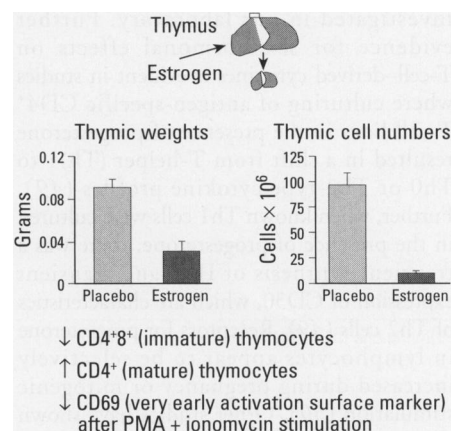


Figure 1. Estrogen effects on thymus. Normal orchiectomized C57BL/6 mice were given estrogen or sham treatment (as a subcutaneous implants of silastic capsules for 3–6 months). Thymuses of these mice were isolated and weighed, and thymocytes were enumerated. Thymocytes were stained with phycoerythrin-anti-CD4 and fluorescein isothiocyanate-anti-CD8 antibodies to determine relative numbers of single-positive (CD4⁺ or CD8⁺) and double-positive (CD4⁺CD8⁺) cells by flow cytometry. In some studies thymocytes were stimulated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin and 8–9 hr later the cell surface expression of CD69 determined. Bar indicates standard error.

hazard. Concanavalin A (ConA)-stimulated T cells from estrogen-treated mice have an early defect, as they have decreased expression of CD69 and have decreased ornithine decarboxylase activity (an early enzyme in polyamine biosynthesis) compared to that in controls.

Sex hormones can affect autoimmune diseases by modulating the activity of CD4⁺ helper cells. The effects of estrogen on CD4⁺ cells are best depicted in studies on β_2 -microglobulin-deficient mice infected with lymphocytic choriomeningitis virus. Females were more susceptible to fatal CD4-mediated meningitis compared to males (45). Estrogen treatment of orchietomized mice rendered these males susceptible to meningitis. *In vitro* studies confirmed that CD4-mediated cytotoxicity was dependent upon estrogen.

Sex hormones could affect the type or levels of T-cell-derived cytokines. In our studies on estrogen-treated normal C57BL/6 mice, splenic lymphocytes stimulated with T-cell stimulants had increased IFN- γ mRNA as well as IFN- γ protein (46). Addition of estrogen to CD4⁺ T-cell lines from multiple sclerosis patients resulted in increased production of IL-10 and IFN- γ (47). Sex hormones could have differential effects on lymphocytes from autoimmune patients compared to those of controls, as was noticed in IL-1 and IL-6 production in lymphocytes from healthy and rheumatoid arthritis patients, which were cultured with estrogen (48). Similarly, it is plausible that endocrine disruptors could have differential effects on normal and autoimmune individuals, an aspect currently investigated in our laboratory. Further evidence for sex hormonal effects on T-cell-derived cytokines is evident in studies where culturing of antigen-specific CD4⁺ T-cell lines in the presence of progesterone resulted in a shift from T-helper (Th)1 to Th0 or Th2-type cytokine profiles (49). Further, when known Th1 cells were cultured in the presence of progesterone, there was a transient synthesis of IL-4 and transient expression of CD30, which are characteristics of Th2 cells (49). Receptors for progesterone in lymphocytes appear to be selectively increased during pregnancy or mitogenic stimulation (50). Other studies have shown that lymphocytes cultured in the presence of progesterone produce an immunomodulatory protein that selectively induces IL-10, IL-4, and IL-3 production by ConA-stimulated lymphocytes (51). Th2-type cytokines secreted by placental tissues are thought to play an important role in maintenance of pregnancy. Is the pregnancy loss noticed in SLE patients (who manifest altered levels or metabolism of sex hormones) due to shifts in the hormone-induced cytokine balance (possibly to Th1 type)? This challenging question warrants further studies.

Estrogenic Compounds, B-Cell Hyperactivity, and Autoantibodies

Since estrogen drastically reduces the size of the bone marrow cavity and the bone marrow cellularity (and thymus), organs where deletion of autoreactive cells occur, it is likely that lymphocytes such as B cells may develop in alternative sites where there is less stringent selection (Figure 2). We thus envisioned the possible appearance of autoreactive cells in the peripheral tissues (e.g., spleen) and expression of autoantibodies in the serum of estrogen-treated mice. We observed that estrogen-treated mice have extensive hemopoietic centers in the liver and spleen (40). Estrogen treatment of MRL/lpr/lpr mice resulted in the appearance of forbidden clones in the liver (52). These cells include $\alpha\beta$ TCR^{Intermediate}, V β 3⁺, or V β 8⁺ T cells that often are deleted in the thymus. Our studies show, by ELISpot and image cytometry, that estrogen activates B cells to produce higher numbers of not only immunoglobulin-producing cells but also autoantibody-producing cells in the spleen which were directed against double-stranded DNA (dsDNA), cardiolipin, phosphatidylserine, and actin (53). The number of plasma cells was significantly increased in the spleens of estrogen-treated mice, suggesting B-cell hyperactivity. Further, sera of estrogen-treated normal C57BL/6 mice had IgM and IgG (predominantly IgG2b) autoantibodies against dsDNA (39), cardiolipin, phosphatidylserine, and phosphatidylinositol (37–39). Although the pathogenic significance of these estrogen-induced autoantibodies is not yet determined, these studies may be important, as anti-dsDNA antibodies are often seen in patients with SLE and antiphospholipid antibodies are present in antiphospholipid syndrome and a subset of SLE patients. Our findings show that treatment of normal mice solely with estrogen can override B-cell tolerance and promote autoimmunity. It is likely that endocrine-disrupting chemicals can also similarly promote B-cell hyperactivity. Our recent preliminary data on mice prenatally exposed to DES also suggest that B cells are modulated by these estrogenic compounds. It is not clear how estrogens (and, by analogy, environmental estrogens) promote B-cell hyperactivity. Conjectural possibilities include a direct effect on the immune system or an indirect effect via the hypothalamic–pituitary axis. It is likely that estrogen may act on T cells to elaborate cytokines (e.g., Th2 cytokines), which in turn promote the function of B cells. Estrogen may downregulate natural killer cells or T-suppressor cells that regulate B-cell functions. B cells from estrogen-treated mice may be readily activated by autoantigens (which may be available following increased hormone-promoted cell death).

Although not conclusively proven, infectious agents in the environment have long been suspected to play a role in the onset or progression of autoimmune diseases. It is thus conceivable that estrogen and possibly environmental estrogens could activate a latent infection that in turn could influence the onset or course of autoimmune diseases.

Estrogenic Chemicals and Apoptosis

Estrogenic compounds can conceivably affect the immune and autoimmune responses by directly or indirectly regulating the apoptotic patterns of lymphocytes. This should not be surprising considering that related steroid hormones such as glucocorticoids directly affect apoptosis of thymocytes. Further, estrogenic hormones affect apoptosis of nonlymphoid cells. For example, depletion of estrogen can

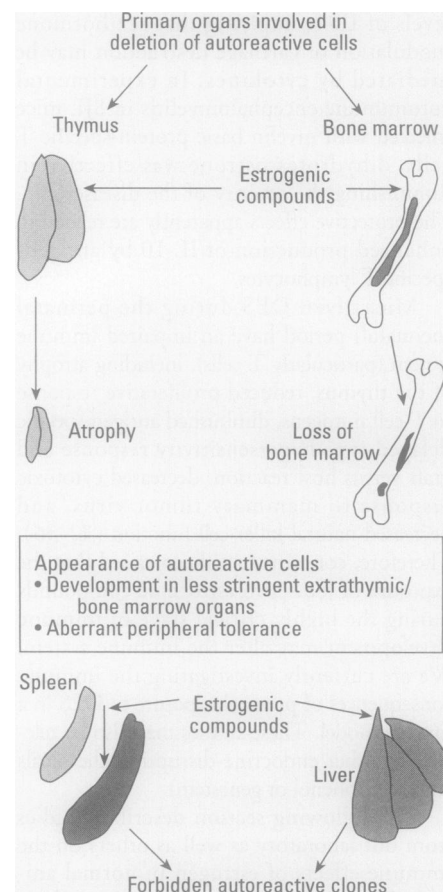


Figure 2. Estrogen-induced alterations in lymphoid organs. Estrogen induces marked atrophy of the thymus and bone marrow. These two primary lymphoid organs are principally involved in deletion of autoreactive cells. Because estrogen profoundly affects these two vital lymphoid organs, it is hypothesized that there may be abnormal regulation of the peripheral tolerance and/or development in extrathymic/bone marrow organs (e.g., liver), where the selection and deletion mechanisms may be less stringent. These events may lead to the appearance of autoreactive cells in the peripheral lymphoid tissues (e.g., spleen).

induce apoptosis in the uterine epithelium (54). In breast tissue, however, estrogen is thought to contribute to the pathology by promoting the survival of estrogen-responsive cancer cells, which is related to *bcl-2* mRNA and protein expression (55). Recent studies in our laboratory suggest estrogen also modulates the apoptosis of lymphocytes (40,56). By several methods, including flow cytometric analysis of cells stained with PI, 7-AAD, and Annexin, we find that spleens from estrogen-treated mice have increased numbers of apoptotic splenic lymphocytes. We are currently investigating whether lymphocytes from estrogen-treated mice have an altered expression of *Bcl-2* or *fas* proto-oncogenes. Splenic lymphocytes from placebo-treated but not from estrogen-treated mice, when activated with T-cell stimulants (e.g., anti-CD3 antibodies) were rescued from apoptosis. Lymphocytes exposed to B-cell stimulants were resistant to cell death (57). We also find that lymphocytes from mice prenatally exposed to DES have altered apoptosis in response to certain stimulants (58). There is evidence of apoptosis and autoimmune diseases in patients with Sjögren syndrome. There is an excessive and inappropriate apoptosis of glandular epithelial cells and inhibition of apoptosis in infiltrating lymphocytes (59). *bcl-2* is a potent downregulator of apoptosis in many systems, including peripheral blood of patients with lupus. The sex hormonal modulation of apoptosis through effects on *bcl-2* is a fertile field for future research uniting the three important fields of hormones, oncogenes, and apoptosis.

Conclusions

The fact that various types of organ and nonorgan-specific autoimmune diseases in both humans and experimental animals occur predominantly in females strongly implies that gender plays a major role in the initiation or progression of these disorders. Studies from a number of laboratories have clearly shown that sex steroid hormones markedly modulate many of these disorders. There is a paucity of information, however, that links environmental estrogens (endocrine-disrupting chemicals) with autoimmune diseases. It is conceivable that a subset of the human population may be more susceptible to autoimmune diseases (as opposed to the general population). It is possible that susceptibility to environmental chemical-induced autoimmunity may vary with the genetic background, gender, previous immune status, age and duration of exposure, and immune status at the time of contact with these agents. Chronic exposure (e.g., sustained low dose) may have more adverse effects than acute exposures. Although valuable data to

ascertain the linkage of environmental agents with autoimmune diseases in humans can be obtained (e.g., performing sequential immunological studies in occupational or accidentally exposed populations, or populations living near contaminated sites), there are inherent limitations in performing detailed mechanistic studies in humans. Animal models can be advantageously employed to study a number of these aspects. For example, the availability of several strains of genetic-prone autoimmune and nonautoimmune mice will elucidate the response of normal and autoimmune individuals to a given chemical. Animal models will also permit the performance of studies involving chronic or acute exposure of chemicals during fetal to adult stages of life. Certain ages of life (e.g., fetal, neonatal, or senescent stages of life) may be more susceptible to environmental chemical-induced autoimmunity.

Our studies and those of others show that estrogens induce imbalances in T and B cells. In general, estrogens bring about hypoactivity of T cell subsets and hyperactivity of B cells (3–5,32). This may form the underlying basis for induction of autoimmunity by estrogen. Although estrogen induces hypoactivity of T cells (as in the above experimental systems), it is nevertheless likely that selected subsets of T cells may be errant in functioning (e.g., overproducing certain cytokines or responding aberrantly to T-cell activation signals). Regulatory cells that hold in check autoreactive cells may be affected by hormones. This could promote immune dysregulation including autoimmunity.

It is important to recognize that the effects of sex hormones on the immune system should not be generalized. To illustrate this point, Sullivan (60) has shown that androgens have a stimulatory effect on mucosal immunity in the eye, no effect on the uterus, and an inhibitory effect in the mammary gland. The outcome of immune responses is dependent upon highly complex interactions of sex hormones with several tissues. Estrogen could have either a stimulatory or suppressive effect on the immune system, depending on dose and duration, the age of the individual, local site, activation status of the cells, availability of costimulatory signals, or the expression of receptors. It should therefore not be surprising that environmental estrogens may have the same or varied effects on various autoimmune diseases. It is anticipated that much human and animal data will become available in the near future to address the precise association of environmental agents and autoimmune diseases. Mechanistic studies in animal models (or in humans) may then prove to be very useful in understanding the induction or advancement of disease, and exploring new avenues to correct these conditions therapeutically.

REFERENCES AND NOTES

1. Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune responses and autoimmune responses: mechanisms of sex hormone action. *Am J Pathol* 121:531–559 (1985).
2. Ansar Ahmed S, Talal N. Sex steroids, sex steroid receptors and autoimmune disorders. In: *Steroid Receptors and Disease: Cancer, Autoimmune, Bone, and Circulatory Disorders* (Sheridan PJ, Blum K, Trachtenberg MC, eds). New York:Marcel Dekker, 1988:289–316.
3. Ansar Ahmed S, Talal N. Effect of Sex hormones on immune responses and autoimmune diseases: an update. In: *A Decade of Autoimmunity* (Shoenfeld Y, ed). Amsterdam:Elsevier, 1998:325–329.
4. Ansar Ahmed S, Talal N. Sex hormones and the immune system—Part 2: Animal data. *Bailliere Clin Rheumatol* 4:13–31 (1990).
5. Ansar Ahmed S, Talal N. Importance of sex hormones in lupus. In: *Dubois' Lupus Erythematosus* (Wallace DJ, Hahn BH, eds). Malvern, PA:Lea Fibiger, 1993:148–156.
6. Grossman CJ. Regulation of the immune system by sex steroids. *Endocr Rev* 5:435–454 (1984).
7. Lahita R. Sex hormones and the immune response—human studies. *Bailliere Clin Rheumatol* 4:1–12 (1990).
8. Olsen NJ, Kovacs WJ. Gonadal steroids and immunity. *Endocr Rev* 17:369–384 (1996).
9. Marselos M, Tomatis L. Diethylstilboestrol. I: Pharmacology, toxicology and carcinogenicity in humans. *Eur J Cancer* 28A:1182–1189 (1992).
10. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* 284:878–881 (1971).
11. Marselos M, Tomatis L. Diethylstilboestrol. II: Pharmacology, toxicology and carcinogenicity in experimental animals. *Eur J Cancer* 29A:149–155 (1993).
12. DES Task Force Report. Washington, DC:U.S. Government Printing Office, 1978.
13. McMartin MC, Kenedy KE, Greenspan KA, Alam P, Greiner P, Yam J. Diethylstilbestrol: a review of its toxicity and use as a growth promotor in food-producing animals. *J Environ Pathol Toxicol* 1:279–313 (1978).
14. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL. Assessing environmental chemicals for estrogenicity using a combination of *in vitro* and *in vivo* assays. *Environ Health Perspect* 104:1296–1300 (1996).
15. Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray E, Kaattari S, Lucier G, Luster M, Mac MJ, Maczka C, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect* 104:715–740 (1996).
16. Jacobson DL, Gange SJ, Rose NR, Graham NMH. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* 84:223–243 (1997).
17. Vollenhoven RFV, Engleman EG, McGuire JL. An open study of dehydroepiandrosterone in systemic lupus erythematosus. *Arthritis Rheum* 37:1305–1310 (1994).
18. Olsen N, Kovacs WJ. Case report: testosterone treatment of systemic lupus erythematosus in a patient with Klinefelter's syndrome. *Am J Med Sci* 310:158–160 (1995).
19. Newbold RR, Bullock BC, McLachlan JA. Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. *Cancer Res* 50:7677–7681 (1990).
20. Noller KL, Blair PB, O'Brien PC, Melton LJ III, Offord JR, Kauffman RH, Colton T. Increased occurrence of autoimmune disease among women exposed in utero to diethylstilbestrol. *Fertil Steril* 49:1080–1082 (1988).
21. Wingard DL, Turiel J. Long-term effects of exposure to diethylstilbestrol. *West J Med* 149:551–554 (1988).
22. Baird DD, Wilcox AJ, Herbst AL. Self-reported allergy, infection and autoimmune diseases among men and women exposed *in utero* to diethylstilbestrol. *J Clin Epidemiol* 49: 263–266 (1996).
23. Vidal S, Gelpi C, Rodriguez-Sanchez JL. (SWR × SJL)_{F1} mice: a new model of lupus-like disease. *J Exp Med* 179: 1429–1435 (1994).
24. Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri PK. Effect of castration and sex hormone treatment on survival, nucleic acid antibodies and glomerulonephritis in NZB × NZW F₁ mice. *J Exp Med* 147:1568 (1978).
25. Walker S., Besch-Williford C., Keisler D. Accelerated deaths from systemic lupus erythematosus in NZB × NZW F₁ mice

- treated with the testosterone-blocking drug flutamide. *J Lab Clin Med* 124:401–407 (1994).
26. Gavalchin J, Shanley P, Silvén CJ, Stoll M, Gasiewicz TA, Silverstone AE. Estradiol (E₂) treatment of male NZB × SWR SNF₁ mice produces immunological alterations associated with lupus-like autoimmune nephritis in female SNF₁ mice. Unpublished abstract presented at the Workshop on Linking Environmental Agents and Autoimmune Diseases, 1–3 September 1998, Research Triangle Park, North Carolina.
 27. Carlsten H, Tarkowski A, Holmdahl R, Nilsson LA. Oestrogen is a potent disease accelerator in SLE-prone MRL/lpr/lpr mice. *Clin Exp Immunol* 80:467–473 (1990).
 28. Dayan M, Zinger H, Kalush F, Mor G, Amir-Zoltman Y, Kohen F, Stohger Z, Moses E. The beneficial effects of treatment with tamoxifen and anti-oestradiol antibody on experimental systemic lupus erythematosus are associated with cytokine modulations. *Immunology* 90:101–108 (1997).
 29. Fitzpatrick F, Lepault F, Homo-Delarche F, Bach J-F, Dardenne M. Influence of castration, alone or combined with thymectomy, on the development of diabetes in nonobese diabetes mouse. *Endocrinology* 129:1382–1390 (1991).
 30. Da Silva JAP, Larbre J-P, Seed MP, Cutolo M, Villaggio B, Scott D, Willoughby DA. Sex differences in inflammation induced cartilage damage in rodents. The influence of sex steroids. *J Rheumatol* 21:330–336 (1993).
 31. Dalal M, Kim S, Voskhl RR. Testosterone therapy ameliorates experimental autoimmune encephalomyelitis and induces a T helper 2 bias in autoantigen-specific T lymphocyte response. *J Immunol* 159:3–6 (1997).
 32. Blair PB. Immunologic consequences of early exposure of experimental rodents to diethylstilbestrol and steroid hormones. In: *Developmental Effects of Diethylstilbestrol (DES) in Pregnancy* (Herbst AL, Bern HA, eds). New York:Thieme-Stratton, 1981;167–178.
 33. Kalland T, Strand O, Forsberg J-G. Long term effects of neonatal estrogen treatment on mitogen responsiveness of mouse splenic lymphocytes. *J Natl Cancer Inst* 63:413–421 (1979).
 34. Kalland T. Exposure of neonatal female mice to diethylstilbestrol persistently impairs NK activity through reduction of effector cells at the bone marrow level. *Immunopharmacology* 7:127–134 (1984).
 35. Kato K, Chen Y, Nakane A, Tomonori M, Fujieda K, Kimura T, Yamamoto K. Suppression of delayed-type hypersensitivity in mice pretreated with diethylstilbestrol. *J Leuk Biol* 43: 530–538 (1988).
 36. Luster MI, Fate RE, McLachlan JA, Clark GC. Effect of in utero exposure to diethylstilbestrol on the immune response in mice. *Toxicol Appl Pharmacol* 47:279–285 (1979).
 37. Ansar Ahmed S, Verthelyi D. Antibodies to cardiolipin in normal C57BL/6: induction by estrogen but not dihydrotestosterone. *J Autoimmun* 6:265–269 (1993).
 38. Verthelyi D, Ansar Ahmed S. 17 β -Estradiol, but not 5 α -dihydrotestosterone, augments antibodies to double stranded deoxyribonucleic acid in non-autoimmune C57BL/6. *Endocrinology* 135:2615–2622 (1994).
 39. Verthelyi D, Ansar Ahmed S. Characterization of estrogen-induced antibodies to cardiolipin in non autoimmune mice. *J Autoimmun* 10:115–125 (1997).
 40. Ansar Ahmed S, Hissong BD, Donner KJ, Nordyke PG, Vaught T, Becker KM, Gogal RM, Walsh JE, Verthelyi DI. Immunological consequences of exposure to estrogens: aberrant spontaneous and induced lymphocyte activation [Abstract]. *Am J Repr Immunol* 40:281 (1998).
 41. Donner KJ, Becker KM, Hissong BD, Ansar Ahmed S. Comparison of multiple assays for kinetic detection of apoptosis in thymocytes exposed to dexamethasone or diethylstilbestrol. *Cytometry* 35:1–11 (1999).
 42. Staples EJ, Fiore NC, Frazier DE, Gasiewicz TA, Silverstone AE. Overexpression of the anti-apoptotic oncogene, bcl-2, in the thymus does not prevent thymic atrophy induced by estradiol or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol Appl Pharmacol* 151:200–210 (1998).
 43. Gulino A, Screpanti I, Torrisi MR, Frati L. Estrogen receptor and estrogen sensitivity of fetal thymocytes are restricted to blast cells. *Endocrinology* 117:47–54 (1985).
 44. Hissong BD, Donner K, Karpuzoglu-Sahin E, Ansar Ahmed S. Unpublished observations.
 45. Muller D, Chen M, Vikingsson A, Hildeman D, Pederson K. Oestrogen influences CD4⁺ T-lymphocyte activity in vivo and in vitro in β -microglobulin deficient mice. *Immunology* 86: 162–167 (1995).
 46. Karpuzoglu-Sahin E, Yin Z, Ansar Ahmed S, Sriranganathan N. Long-term estrogen treatment alters cytokine gene and protein expression in normal mice [Abstract]. *Exp Biol* 99:A320 (1999).
 47. Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* 158:446–451 (1997).
 48. Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells *in vitro*. *Clin Exp Rheumatol* 11:157–162 (1993).
 49. Piccinni M-P, Giudizi M-G, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C, et al. Progesterone favors the development of human T helper cells producing Th2-type cytokine and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. *J Immunol* 155: 128–133 (1995).
 50. Szekeres-Bartho J, Szekeres GY, Debre P, Autran B, Chaoaut G. Reactivity of lymphocytes to a progesterone receptor-specific monoclonal antibody. *Cell Immunol* 125: 273–283 (1990).
 51. Szekeres-Bartho J, Wegmann TG. A progesterone-dependent immunomodulatory protein alters the Th1/Th2 balance. *J Reprod Immunol* 31:81–95 (1996).
 52. Okuyama R, Abo T, Seki S, Ohteki T, Sugiura K, Kusumi A, Kumagai K. Estrogen administration activates extrathymic T cell differentiation in the liver. *J Exp Med* 175:661–669 (1992).
 53. Verthelyi D, Ansar Ahmed S. Estrogen increases the number of plasma cells and enhances their autoantibody production in non-autoimmune C57BL/6 mice. *Cell Immunol* 189: 125–134 (1998).
 54. Jo T, Terada N, Saji F, Tanizawa O. Inhibitory effects of estrogen, progesterone, androgen and glucocorticoid on death of neonatal mouse uterine epithelial cells induced to proliferate by estrogen. *J Steroid Biochem Mol Biol* 46: 25–32 (1993).
 55. Teixeira C, Reed JC, Pratt M. Estrogen promotes chemotherapeutic drug resistance by a mechanism involving Bcl-2 proto-oncogene expression in human breast cancer cells. *Cancer Res* 55:3902–3907 (1995).
 56. Verthelyi D. Effects of Estrogen on the B-Cell Functions of Normal Mice. PhD Thesis. Blacksburg, VA:Virginia Polytechnic Institute and State University, 1996.
 57. Verthelyi D, Ansar Ahmed S. Unpublished observations.
 58. Hissong BD, Donner KJ, Vaught T, Nordyke P, Ansar Ahmed S. Unpublished observations.
 59. Kong L, Ogawa N, Nakabayashi T, Liu GT, D-Souza E, McGuff HS, Guerrero D, Talal N, Dang H. Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 40:87–97 (1997).
 60. Sullivan DA. Hormonal influence on the secretory immune system of the eye. In: *The Neuroendocrine-Immune Network* (Frier S, ed). Boca Raton, FL:CRC Press, 1990;200–237.